

Supplementary Table 1 Participants clinical data.

PTID	Sex	Plasma viral load (copies/mL ⁻¹)	CD4 count (mL ⁻¹)	On ART	
LTNP-1	M	792	570	no	
LTNP-2	M	<50	522	no	
LTNP-3	M	<50	473	no	
LTNP-4	F	800	421	no	
LTNP-5	M	<50	796	no	
LTNP-6	M	<50	832	no	
LTNP-7	M	125	783	no	
LTNP-8	M	<50	759	no	
LTNP-9	F	<50	773	no	
LTNP-10	M	65	426	no	
LTNP-11	F	<50	643	no	
LTNP-12	F	1073	574	no	
LTNP-13	M	140	555	no	
LTNP-14	M	<50	632	no	
LTNP-15	M	540	578	no	
LTNP-16	M	990	550	no	
LTNP-17	M	<50	602	no	
LTNP-18	M	<50	575	no	
LTNP-19	F	800	451	no	
LTNP-20	M	<50	790	no	
LTNP-21	M	<50	850	no	
LTNP-22	M	125	783	no	
LTNP-23	M	<50	765	no	
LTNP-24	M	4500	562	no	
ART-1	M	<30	720	yes	
ART-2	M	<30	570	yes	
ART-3	M	<30	1080	yes	
ART-4	M	<30	450	yes	
ART-5	M	1399	800	yes	
ART-6	M	<30	720	yes	
ART-7	M	<30	830	yes	
ART-8	M	<30	460	yes	
ART-9	M	<30	470	yes	
ART-10	F	<30	410	yes	
ART-11	M	<30	350	yes	
ART-12	M	<30	850	yes	
ART-13	M	<30	740	yes	
ART-14	M	<30	680	yes	
ART-15	M	<30	470	yes	

ART-16	M	<30	550	yes	
ART-17	M	208	410	yes	
ART-18	M	<30	420	yes	
ART-19	M	<30	580	yes	
ART-20	F	<30	590	yes	
ART-21	M	<30	680	yes	
ART-22	M	<30	570	yes	
ART-23	M	<30	420	yes	
ART-24	M	<30	210	yes	
ART-25	M	<30	500	yes	
ART-26	M	<30	640	yes	
ART-27	M	<30	260	yes	
ART-28	F	<30	700	yes	
ART-29	F	<30	720	yes	
ART-30	M	<30	910	yes	
ART-31	F	168	290	yes	
ART-32	F	<30	180	yes	
ART-33	M	<30	680	yes	
ART-34	M	<30	510	yes	
ART-35	M	<30	1210	yes	
ART-36	F	<30	550	yes	
ART-37	F	60	910	yes	
ART-38	F	<30	190	yes	
ART-39	M	<30	340	yes	
ART-40	F	160	60	yes	
ART-41	M	<30	390	yes	
ART-42	M	<30	250	yes	
ART-43	M	<30	520	yes	
ART-44	M	<30	610	yes	
ART-45	M	<30	360	yes	
ART-46	M	<30	290	yes	
ART-47	M	<30	250	yes	
ART-48	M	<30	260	yes	
AET-49	M	<30	342	yes	
ART-50	F	<30	457	yes	

Healthy control (HC)

Long-term non-progressor (LTNP)

On antiretroviral therapy (ART)

Male (M) and female (F)

The mean age for HCs was 37.5 (range 22-62), 21 males and 11 females.

Supplementary Table 2.

PTID	Sex	Age
HC-1	M	43
HC-2	M	33
HC-3	M	33
HC-4	M	47
HC-5	M	55
LTNP-1	F	53
LTNP-2	M	41
LTNP-3	M	34
LTNP-4	M	49
LTNP-5	M	34
ART-1	M	42
ART-2	M	35
ART-3	M	36
ART-4	M	46
ART-5	M	54

Tregs from these subjects were used for the RNAseq analysis.

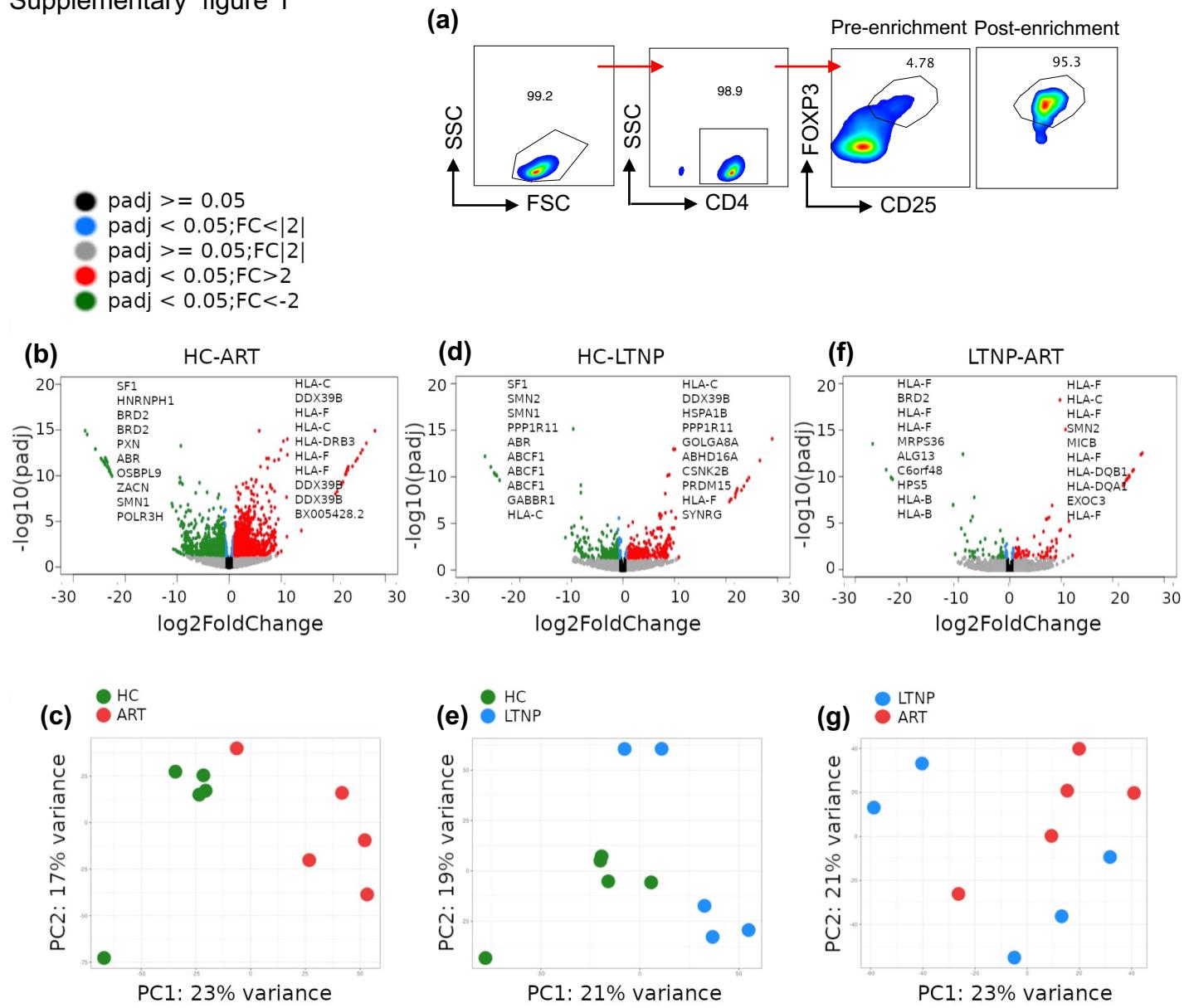
Healthy control (HC)

Long-term non-progressor (LTNP)

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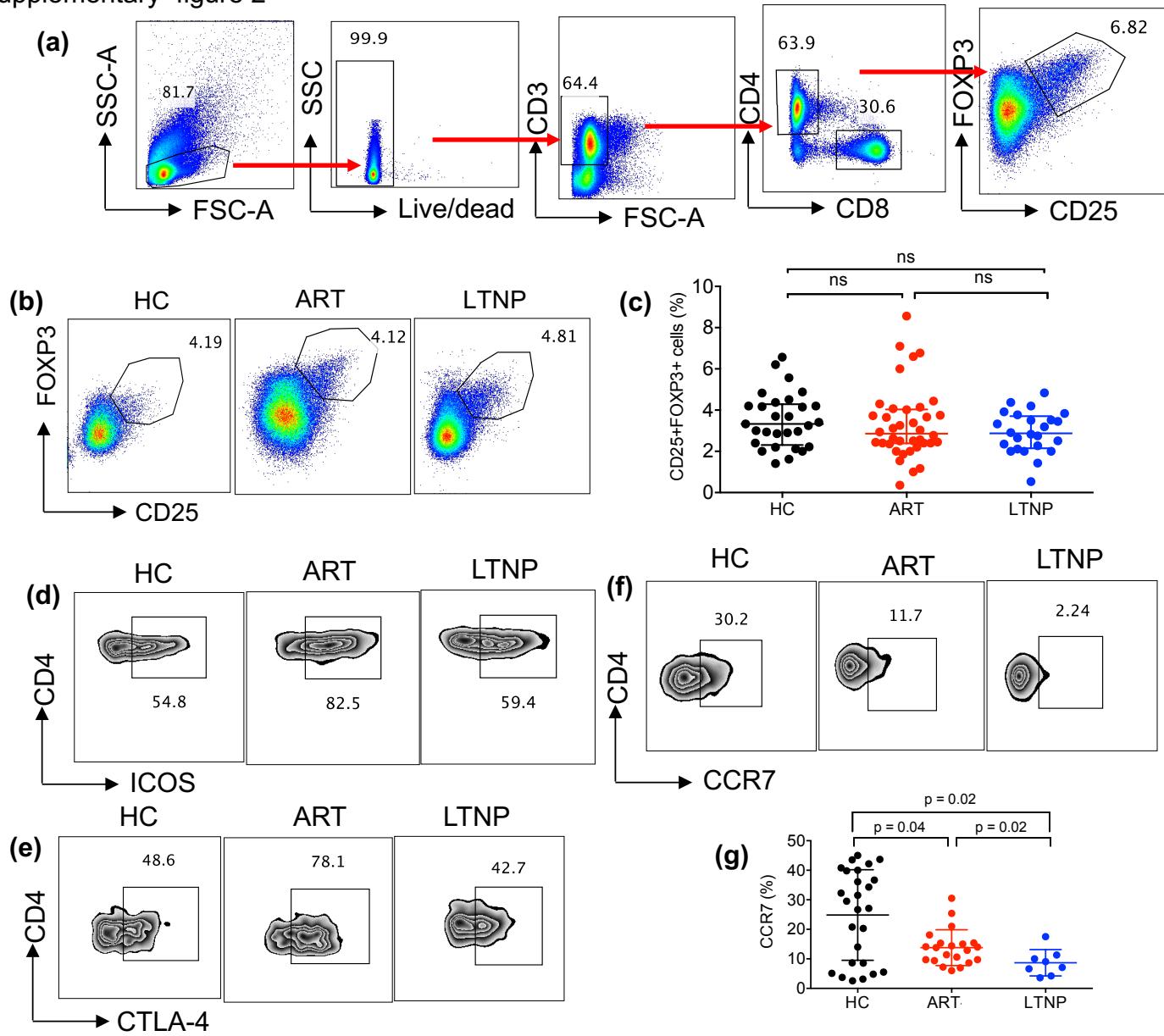
Male (M) and female (F)

Supplementary figure 1



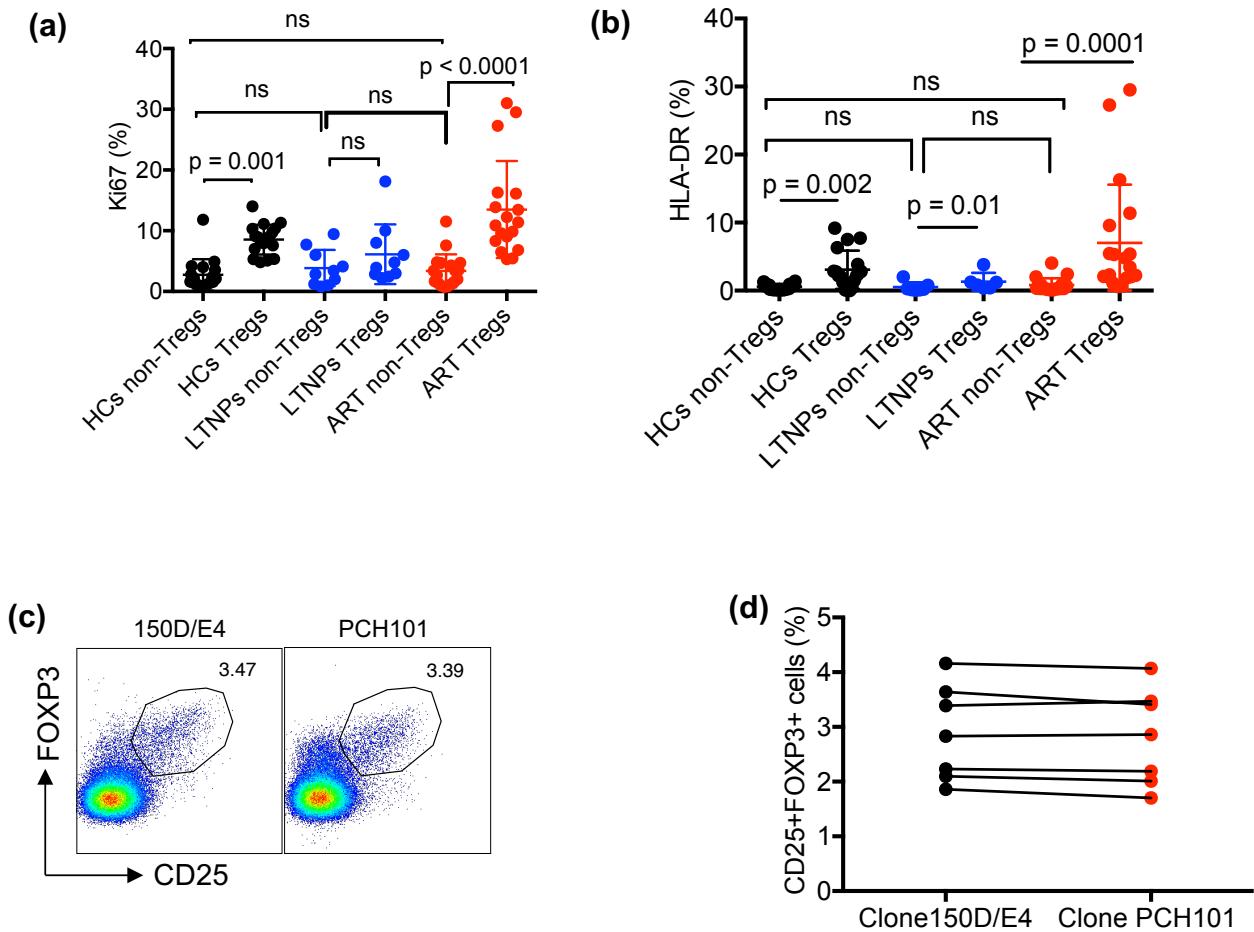
Supplementary figure 1. (a) Representative flow cytometry plots showing the purity of Tregs pre-and post-enrichment. (b), (d), (f) MA plots depicting abundance, fold-change and significance of transcripts in Tregs of different indicated groups. Black dots represent transcripts that were not differentially expressed (DE) between different groups. Grey dots represent DE transcripts with a $-2 < \text{fold-change} < 2$. Red dots represent DE transcripts with a fold-change > 2 . Green dots represent DE transcripts with a fold-change < -2 . (c), (e), (g) Principal component analysis (PCA) on the Euclidian distances between Tregs of different study cohorts. HC (healthy control), ART (Anti-retroviral treated patients) and LTNP (long-term non-progressors). From one RNAseq analysis ($n=5/\text{group}$).

Supplementary figure 2



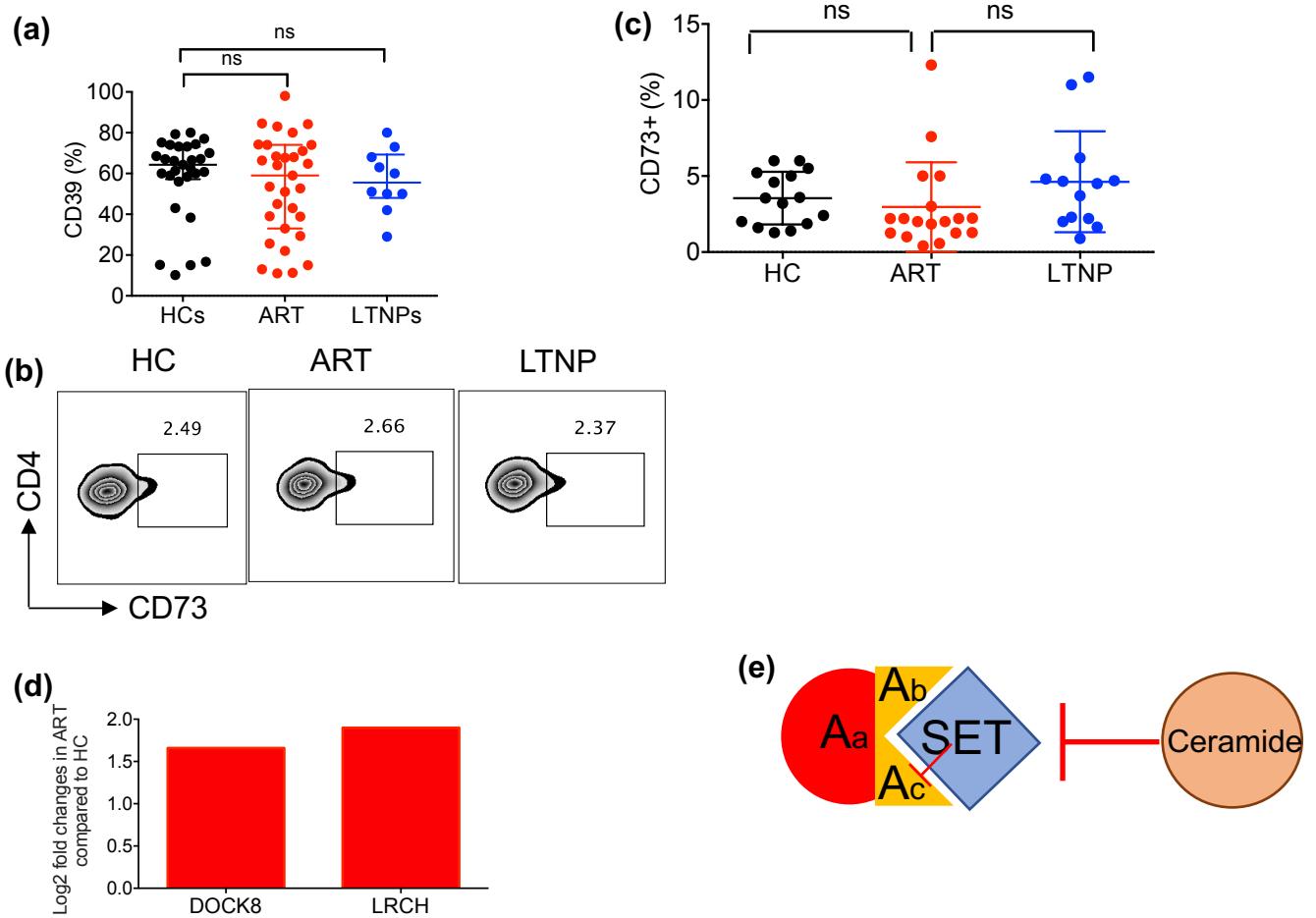
Supplementary figure 2. **(a)** Representative plots of gating strategy for Tregs identification. **(b)** Representative flow cytometry plots, and **(c)** cumulative data of Tregs frequency in different study groups, (n=38 HC, n=50 ART, n=24 LTNP). **(d)** Representative flow cytometry plots of ICOS expression in Tregs of different study groups. **(e)** Representative flow cytometry plots of CTLA-4 expression in Tregs of different study groups. **(f)** Representative flow cytometry plots, and **(g)** cumulative data of CCR7 expression in Tregs of different study groups (n=26 HC, n=21 ART, n=8 LTNP). Data are obtained from 3 or 4 independent experiments.

Supplementary figure 3



Supplementary figure 3. **(a)** Cumulative data showing percentage of Ki67, and **(b)** HLA-DR expressing Tregs versus non-Tregs-CD4⁺ T cells in HC (n=18), ART (n=18) and LTNP (n=10). **(c)** Representative flow cytometry plots, and **(d)** cumulative data of percentages of Tregs in the same HIV-infected individuals comparing two different clones for the anti-FOXP3 antibody (n=7). Data are obtained from 2 or 3 independent experiments.

Supplementary figure 4



Supplementary figure 4. **(a)** Cumulative data showing percentage of CD39⁺ Tregs in different study cohorts (n=28 HC, n=31 ART, n=10 LTNP). **(b)** Representative flow cytometry plots, and **(c)** cumulative data of intracellular CD73 expression in Tregs of different study groups (n=15 HC, n=18 ART, n=13 LTNP). **(d)** Log2 fold change of DOCK8 and LRCH genes in Tregs of the ART-T group compared to HCs. **(e)** Simplified schematic of the effects of ceramide on the function of PP2A holoenzyme subunits via SET oncoprotein. Each dot represents a human subject either HC or HIV-infected. Data are obtained from 3 to 5 independent experiments. RNAseq data are from 5 samples/group.

Supplementary Table 3 (Fig 3d)

Gene name	Log2 Fold change in ART compared to HC	P value
Cytotoxic T-lymphocyte associated protein 4 (CTLA-4)	1.25	0.001
Transferrin receptor (CD71)	2	0.0006
NGFI-A binding protein 2 (Nab2)	2.21	0.003
Nuclear factor of activated T cells 1 (Nfatc1)	2.97	0.0005
Interferon regulatory factor 4 (IRF-4)	3.47	9.05501E-07
Nuclear receptor subfamily 4 group A member 1 (Nr4a1)	3.9	0.002
CD44	5	0.0005

Supplementary Table 4 (Fig 3e)

Gene name	Log2 Fold change in ART compared to HC	P value
Cytotoxic T-lymphocyte associated protein 4 (CTLA-4)	1.25	0.001
Kinesin family member 23 (Kif23)	1.35	0.002
T cell immunoreceptor with Ig and ITIM domains (TIGIT)	1.47	4.85129E-05
Protein tyrosine phosphatase, receptor type J (PTPRJ)	2	5.97522E-06
Ataxin 1 (ATXN1)	2.2	3.91563E-05
Dual specificity phosphatase 16 (DUSP16)	2.28	0.000856893

C-C motif chemokine ligand 22 (CCL22)	2.48	5.83422E-05
Pleckstrin homology domain containing B2 (PLEKHB2)	2.58	0.000479517
CD25	2.93	0.000462961
G protein subunit gamma 2 (Gng2)	7.32	3.92287E-05

Supplementary Table 5 (Fig 3F)

Gene name	Log2 Fold change in ART compared to HC	P value
Transcription factor 7 (TCF7)	-1.3	0.000142302
RELB proto-oncogene, NF- κ B subunit (RELB)	1.35	0.000842881
C-C motif chemokine receptor 7 (CCR7)	-1.7	6.9175E-05
RELA proto-oncogene, NF- κ B subunit (RELA)	-2.5	0.000495637
Lymphoid enhancer binding factor 1 (LEF1)	-23.43	1.62451E-15

Supplementary Table 6 (Fig 4B)

Gene name	Log2 Fold change in ART compared to HC	P value
Ribosomal protein S3A (RPS3A)	3	3.57916E-06
Ribosomal protein S27 (RPS27L)	1.32	9.78056E-05
Ribosomal protein S15a (RPS15A)	1.12	0.000136744
Ribosomal protein S24 (RPS24)	5.94	0.00439609

Ribosomal protein S16 (RPS16)	1.1	2.2405E-05
Ribosomal protein S14 (RPS14)	-1.75	0.002522177
Ribosomal protein S6 (RPS6)	1.77	3.76778E-07
Ribosomal protein S3 (RPS3)	1.13	0.003304774
Ribosomal protein lateral stalk subunit P2 (RPLP2)	2	0.000125663
Ribosomal protein lateral stalk subunit P1 (RPLP1)	1.35	4.04459E-10
Ribosomal protein L39 like (RPL39L)	4.13	0.002418652
Ribosomal protein L18a (RPL18A)	1.46	9.05286E-05
Ribosomal protein L39 (RPL39)	2	0.000111302
Ribosomal protein L35 (RPL35)	1.68	0.000147745
Ribosomal protein L34 (RPL34)	1.48	0.000495182
Ribosomal protein L32 (RPL32)	1.27	8.2931E-05
Ribosomal protein L30 (RPL30)	-1.47	0.000734192
Ribosomal protein L28 (RPL28)	1.86	1.82958E-05
Ribosomal protein L14 (RPL14)	2.13	1.43181E-11
Ribosomal protein L5 (RPL5)	1.9	6.34523E-08
Ribosomal protein L4 (RPL4)	-1.3	0.002013026

Supplementary Table 7 (Fig 4C)

Gene name	Log2 Fold change in ART compared to HC	P value
Eukaryotic translation initiation factor 2A	1.7	0.002615925

Eukaryotic translation initiation factor 2 alpha kinase 2	2.73	0.003415744
Eukaryotic translation initiation factor 5	2.17	0.00313122
Ribosomal protein S6 kinase B1	2.22	5.60961E-05

Supplementary Table 8 (Fig 4G)

Gene name	Log2 Fold change in ART compared to HC	P value
Transforming growth factor beta receptor 2 (TGFBR)	-1.3	0.00032547
AHNAK nucleoprotein (AHNAK)	1.48	0.001762867
ADP ribosylation factor like GTPase 4C (ARL4C)	-1.5	0.000832051
BCL-9	-1.67	0.000281316
Rho guanine nucleotide exchange factor 12 (ARHGEF12)	1.6	2.2405E-05
FERM domain containing 4B (FRMD4B)	1.88	0.000216146
RAS guanyl releasing protein 2 (RASGRP2)	-2	0.000599225
Transmembrane inner ear (TMIE)	-2.25	0.003353353
Solute carrier family 2 member 3 (SLC2A3)	2.32	0.000711445
MAX dimerization protein 1 (MXD1)	2.35	0.00319058

Protein tyrosine phosphatase, receptor type S (PTPRS)	2.38	0.002341638
Unc-119 lipid binding chaperone (UNC119)	2.6	5.5049E-05
ATPase phospholipid transporting 8B4 (putative) (ATP8B4)	-2.66	0.000572542
Cytokine inducible SH2 containing protein (CISH)	3.2	1.72531E-06
Inositol polyphosphate-5-phosphatase F (INPP5F)	3.21	7.85463E-06
Sprouty RTK signaling antagonist 1 (SPRY1)	4.9	1.62247E-05
Prolyl 4-hydroxylase subunit alpha 1 (P4HA1)	5.9	0.000854751
lymphoid enhancer binding factor 1 (LEF1)	-23.4	1.62451E-15